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Optimization of Saponin Extract from Red Sage (*Salvia miltiorrhiza*) Roots Using Response Surface Methods and Its Antioxidant and Anticancer Activities

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Abstract

Red sage (*Salvia miltiorrhiza* Bunge) is a perennial herb containing various bioactive compounds that promote human health. In this study, single-factor experiments were first conducted, followed by the optimization of extraction conditions to maximize the saponin content from red sage root extracts. In the single-factor experiments, the highest saponin content (47.5 ± 0.88 mg/g) was obtained using 80% ethanol, a solvent-to-material ratio of 40:1 (mL/g), an extraction period of 3 h, and an extraction temperature of 60 °C. Response Surface Methodology (RSM) was performed to optimize the extraction parameters with a material-to-solvent ratio of 41.31:1 (mL/g), an extraction temperature of 58.08 °C, and an extraction time of 3.16 h. Under these optimized conditions, the experimental saponin content reached 47.71 ± 0.15 mg/g. Additionally, crude extract of red sage exhibited antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals with an IC_{50} value of 16.24 µg/mL. This extract also demonstrated anticancer against 61.79 ± 3.57% HepG2 cancer cells at a concentration of 100 µg/mL.

Keywords: saponin; RSM; *Salvia miltiorrhiza*; antioxidant; anticancer; HepG2

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1. Introduction

Oxidative stress is a common pathological mechanism underlying various chronic diseases, including cancer and cardiovascular disorders, and has drawn increasing interest in natural antioxidant strategies such as the use of plant-based compounds [1]. In spite of the wide use of synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene, there are concerns about their long-term safety and carcinogenicity. In response to this, natural alternatives have gained interest [2,3].

Plant-derived saponins present a promising avenue for both research and commercial applications due to their exceptional physicochemical and biological properties. Their natural origin makes them eco-friendly, biodegradable, and non-toxic, which is of paramount importance from environmental and health perspectives [4]. In recent years, the extraction of saponins from medicinal plants has gained increasing attention as a promising approach to obtaining bioactive compounds for pharmaceutical applications [5]. In particular, extracts enriched in saponins from medicinal plants are being explored as multifunctional agents

with antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, and immunomodulatory properties [6,7]. They have also been proposed as promising lead compounds for the development of functional foods or botanical-based pharmaceuticals. They are particularly effective in liver cancer models like the HepG2 cell line, where they induce apoptosis and reduce oxidative stress. The HepG2 cell line is frequently used in anticancer and hepatotoxicity screening due to its well-characterized genomic profile and stable phenotype, making it a reliable in vitro model for evaluating the bioactivity of natural compounds [8].

Salvia miltiorrhiza (red sage or Dan Sam) is a traditional Chinese medicinal plant commonly used for cardiovascular treatment [9]. *S. miltiorrhiza* contains several natural metabolites, which have been systematically characterized. These include phenolic acids (salvianic acid, rosmarinic acid, salvianolic acid A, salvianolic acid B), lipophilic diterpenes (tanshinone I, tanshinone IIA, tanshinone IIB, tanshinone VI, dihydrotanshinone I, cryptotanshinone, tetrahydrotanshinone), and triterpenoid saponins (ursolic acid) [10]. These compounds have demonstrated significant pharmacological effects relevant to human health [11]. For instance, ursolic acid saponins have been specifically identified for their antioxidant, anti-inflammatory, and anticancer properties [12,13]. Similarly, tanshinone IIA, a major lipophilic compound, promotes cell death by thiol depletion, mitochondrial disruption, and ROS generation [14]. Ethanol and acetone extracts of *S. miltiorrhiza* roots further confirm time- and dose-dependent cytotoxicity and apoptotic effects in HepG2 cells, as shown by increased LDH leakage and reduced ATP levels [15].

However, the efficient extraction of saponins remains challenging, as yield and reproducibility depend on multiple interacting variables including solvent polarity, extraction temperature, solid–liquid ratio, and duration. Response Surface Methodology (RSM) is a useful multivariate statistical method that has been proven to work well for studying complex extraction processes. RSM enhances the precision of predictions and facilitates the identification of optimal conditions. This is achieved by systematically examining the individual and interactive effects of multiple factors, thereby significantly reducing the number of experiments required [16].

Previous studies have successfully applied RSM to optimize the extraction of saponins from other medicinal plants such as *Panax notoginseng* [17] and *Camellia sinensis* [18], demonstrating its suitability for saponin-rich systems. However, to the best of our knowledge, no prior study has optimized the extraction of saponins from *S. miltiorrhiza* using RSM. This represents a novel and practical research direction that can contribute to significantly advancing the recovery methods for saponin extraction.

Therefore, this study was carried out with three main goals: (i) to first explore the effects of different extraction factors on the total saponin content of *Salvia miltiorrhiza* roots using single-factor experiments; (ii) to optimize the extraction process by applying the Box–Behnken Design (BBD) through RSM; and (iii) to test the biological activities of the optimized extract, including its antioxidant ability using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test and its cytotoxic effects on HepG2 liver cancer cells.

2. Materials and Methods

2.1. Chemicals

Methanol (MeOH, 99.5%), acetone (99.5%), ethanol (EtOH, 99.5%), Ethyl acetate (EtOAc, 99.5%), oleanolic acid (97%), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (Singapore). Acetic acid (96%), vanillin, and perchloric acid (70%) were purchased from Merck (Darmstadt, Germany).

2.2. Plant Samples

Red sage (*Salvia miltiorrhiza*) root powder was acquired from Thanh Binh Herb Co., Ltd. Company (Ho Chi Minh City, Vietnam). The powder was dried to obtain constant weight, with a moisture concentration of 9.28 ± 0.07%, and stored at room temperature for further experiments.

2.3. Effect of Solvent Extraction on Saponin Concentration

To examine the influence of solvents on the saponin extraction from *S. miltiorrhiza* roots, an aliquot of 50 mL each of five different solvents with different polarity, including 70% MeOH, 70% EtOH, 70% acetone, 70% EtOAc, and distilled water (DW), were mixed with 5 g of plant material at 60 °C for 3 h [19,20]. The extraction layers were collected through filtration and dried by a rotary evaporator (N-1110, EYELA Co., Tokyo, Japan) at 45 °C for TSC determination.

2.4. Single-Factor Effectes on Saponin Concentration

Prior to RSM, preliminary experiments were conducted to establish appropriate ranges for independent variables. Individual effects of the solvent concentrations (50, 60, 70, 80, and 90%), solvent-to-material ratios (10:1, 20:1, 30:1, 40:1, and 50:1 mL/g), extraction temperatures (40, 50, 60, and 70 °C), and extraction times (2, 2.5, 3, 3.5 and 4 h) were systematically evaluated based on total saponin content.

2.5. Experiment Design and Optimization

The extraction of saponins was optimized using the Box–Behnken model—RSM including material-to-solvent ratio (A), temperature (B), and time (C). The variables with actual levels in the Box–Behnken model are shown in Table 1. The extract layers of each run were collected and determined TSC using the following equation:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad (1)$$

where Y represents the TSC, material-to-solvent ratio, while A, B and C are ethanol-to-material ratio, temperature, and time, respectively; β_0 is a constant. The linear effects of the variables are represented by coefficients β_1, β_2, and β_3. Quadratic effects are accounted for by coefficients β_{11}, β_{22}, and β_{33}, and interaction effects between variables are captured by coefficients β_{12}, β_{13}, and β_{23}. The regression analysis and analysis of variance (ANOVA) were carried out using Design Expert 13 (Stat-Ease, Minneapolis, MN, USA) to determine the model coefficients, establish the empirical model, and subsequently predict the optimal extraction conditions for maximizing TSC.

Table 1. Independent variables and their corresponding levels.

Variable	Symbol	Level		
		−1	0	1
Ethanol-to-material ratio (mL/g)	A	30	40	50
Temperature (°C)	B	50	60	70
Time (h)	C	2.5	3	3.5

2.6. Total Saponin Content Determination

The standard curve of saponins was established following previous studies [20,21], with slight modification. Oleanolic acid (97%) was used as standard in the quantitative determination of the content of saponin. Five solutions of oleanolic acid with concentrations of 50, 100, 200, 250, and 300 μg/mL in ethanol were mixed with vanillin–acetic acid solution

(5% w/v) and perchloric acid. The mixtures were subsequently incubated for 15 min at 60 °C in a water bath. When the solutions were cooled, a 5 mL aliquot of acetic acid was added. The absorbance of each sample was obtained at 548 nm using a UV-Vis spectrophotometer (Jenway-6705, Staffordshire, UK). The regression equation of the oleanolic acid calibration curve was determined as y = 0.0058x − 0.0602 (R^2 = 0.9948). The total saponin content was calculated as milligrams OAE per gram dried leaf sample (mg OAE/g).

2.7. Antioxidant Activity

The DPPH radical scavenging activity of the extracts was assessed using a slightly modified version of a previous method [19]. An aliquot of 2 mL of 0.1 mM DPPH solution was mixed with 2.5 mL of the extract at several concentrations (4–20 µg/mL). The resulting mixtures were then incubated in the dark for 30 min. Absorbance measurements were taken at 517 nm using a UV/VIS spectrophotometer (Jenway-6705, Staffordshire, UK). The percentage inhibition of the DPPH radical was calculated using the following equation:

$$I(\%) = \left(1 - \frac{A_1}{A_0}\right) \times 100 \quad (2)$$

where A_0 represents the absorbance of DPPH and ethanol, and A_1 demotes the absorbance of mixture of DPPH and extract. The inhibitory concentration at 50% (IC_{50}) was subsequently determined by plotting the DPPH scavenging activity against the sample concentration.

2.8. Anticancer Activity

The anticancer activity of crude extract from red sage roots was determined by sulforhodamine B assay using HepG2 cells, following a previous study [22]. HepG2 cells (HB-8065) purchased from ATCC (Manassas, Rockville, ML, USA) were treated with the extract at a concentration of 100 µg/mL for 48 h in 96-well plates, and then the growth inhibition was measured with the formula I% = (1 − [ODt/ODc] × 100)%, where ODt represents the optical density of the test sample and ODc represents the optical density of the control sample. Camptothecin (Calbiochem) was used as a positive control in this assay.

2.9. Statistical Analysis

Experimental data were expressed as means ± standard error and analyzed using one-way ANOVA followed by Duncan's test, with statistical significance defined at $p \leq 0.05$. The data from the single-factor effect study, DPPH radical scavenging activity assay, and anticancer activity assay were analyzed separately using the statistical software SPSS (version 23.0, SPSS Inc., Chicago, IL, USA). The RSM-BBD model was analyzed by Design-Expert 13 (Stat-Ease, Minneapolis, MN, USA).

3. Results and Discussion

3.1. Solvent Screening

A total of five different solvents, including EtOH 70%, MeOH 70%, acetone 70%, EtOAc 70%, and DW, were investigated for their effect on total saponin concentration extracted from red sage roots (Figure 1). EtOH effectively extracted the highest saponin component from red sage, followed by acetone with TSC, at 39.26 and 35.99 mg/g, respectively. DW exhibited the lowest effect on the extraction of saponins with TSC, at 19.95 mg/g. EtOAc and MeOH moderately extracted saponins with TSC, at levels of 31.43 and 24.92 mg/g, respectively. Also, several previous studies have used EtOH for total saponin extraction optimization due to its environmentally friendly property. Therefore, EtOH was chosen for extracting saponin. These findings align with previous research on *Saponaria cypria*

roots, where acetone extraction resulted in the highest TSC (169.00 mg oleanolic acid equivalents/g crude extract), surpassing ethanol (106.21 mg/g) and methanol (64.33 mg/g) extractions [23].

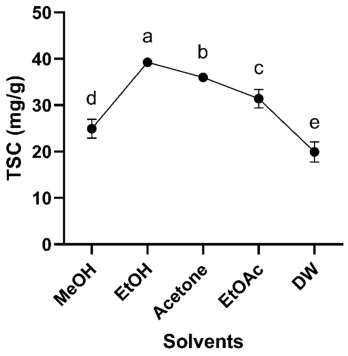


Figure 1. Effect of different solvents on TSC extracted from *Salvia miltiorrhiza* (Red Sage) roots. The experiment was conducted in triplicate (n = 3). Different letters (a–e) indicate a statistically significant difference among five different sovlents.

3.2. Single Factor Experiments

3.2.1. Effect of Solvent Concentration

To determine the effect of solvent concentration on TSC yield, the extraction was performed at 60 °C for 3 h with five different ethanol concentrations in the solvent-to-material ratio. The effect of ethanol concentration is exhibited in Figure 2A. As the ethanol concentration increased from 50 to 90%, the TSC yield increased steadily from 35.16 to 42.12 mg/g. There was no statistically significant difference between ethanol concentrations of 80 and 90%, with TSC yields of 40.57 and 42.12 mg/g dried sample, respectively. Moreover, Gong et al. [24] also mentioned that 79–80% ethanol was found to be the optimal solvent concentration for extracting saponins from *Panax notoginseng*. Thus, 80% ethanol was selected for further experiments.

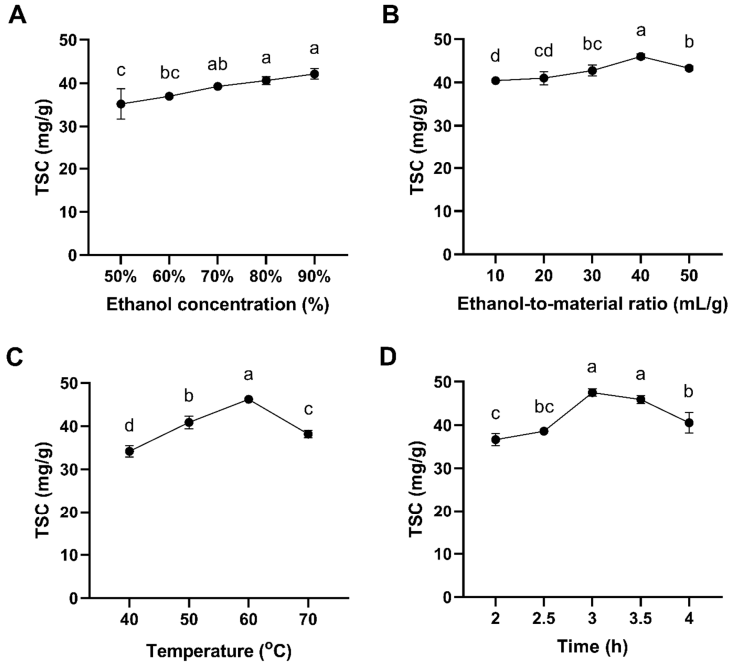


Figure 2. Effects of (**A**) ethanol concentration (**B**) ethanol-to-material ratio, (**C**) extraction temperature, and (**D**) extraction time on TSC extracted from *Salvia miltiorrhiza* roots. These experiments were conducted in triplicate (n = 3). Different letters (a–d) in one graph indicate a statistically significant difference among different levels in one factor.

3.2.2. Effect of Material-to-Ethanol

To investigate the effect of the ethanol-to-material ratio on the TSC yield, the extraction was performed at 60 °C for 3 h using 80% ethanol across five different ratios ranging from 10 to 50 mL/g. As shown in Figure 2B, the TSC yield significantly increased from 40.34 to 45.99 mg/g as the ratio rose from 10 to 30 mL/g. However, when the ratio was further increased to 50 mL/g, the TSC yield decreased to 43.30 mg OAE/g. Consequently, an ethanol-to-material ratio of 40 mL/g was chosen as the baseline level (0) for subsequent experiments.

3.2.3. Effect of Temperature

The effect of extraction temperature on TSC yield is presented in Figure 2C. As the temperature increased from 40 to 60 °C, the TSC yield proportionally rose from 34.16 to 46.19 mg/g. Then, the TSC yield dramatically decreased to 38.16 mg OAE/g when the temperature reached to 70 °C. Higher temperatures generally accelerate the diffusion of compounds, which can lead to increased TSC. Nevertheless, excessively high extraction temperatures can diminish saponin's extraction efficiency, likely due to the decomposition and denaturation of saponins, thereby reducing the overall TSC [20]. Additionally, Khoang, Huyen, Chung, Duy, Toan, Bich, Minh, Pham and Hien [20] also indicated that the optimal temperature for saponin extraction from Polyscias fruticosa roots is 60 °C. Thus, a temperature of 60 °C was selected as the baseline level (0) for subsequent statistical optimization.

3.2.4. Effect of Time

Finally, the extraction time was varied across five different values ranging from 2 to 4 h. The results show that as the extraction time increased from 2 to 3 h, the TSC yield significantly increased from 36.61 to 47.5 mg/g. However, when the extraction time was extended from 3 to 3.5 h, the extraction system reached a dynamic equilibrium. Prolonged extraction times beyond this point led to a decline in the extraction efficiency of saponins and increased processing costs. Hence, an extraction time of 3 h was selected as the baseline level (0) for statistical optimization.

3.2.5. Optimization of Extract Conditions Using RSM

The experimental design matrix was composed of 15 runs with 3 factors (Table 2). The central experiments (run 13–15) were used to determine the experimental error. A quadratic model was employed to express the relationship between the measured response and the input variables, as follows:

$$Y = 46.16 + 0.4037A - 0.3513B + 0.58C + 0.115AB + 0.0925AC - 0.6575BC - 1.54A^2 - 1.41B^2 - 1.06C^2$$

where A, C, AB, and AC factors positively influenced the measured response, whereas the other factors demonstrated a negative effect.

Table 2. BBD for investing simultaneous effects of extraction conditions.

Standard	Runs	Ethanol-to-Material Ratio	Temperature	Time	TSC Yield (mg/g)
11	1	0	−1	1	45.23 ± 1.14 *
3	2	−1	1	0	42.51 ± 0.67
12	3	0	1	1	43.05 ± 0.65
1	4	−1	−1	0	43.28 ± 0.81
6	5	1	0	−1	43.26 ± 0.96
5	6	−1	0	−1	42.45 ± 1.12
14	7	0	0	0	46.47 ± 0.93

Table 2. *Cont.*

Standard	Runs	Ethanol-to-Material Ratio	Temperature	Time	TSC Yield (mg/g)
4	8	1	1	0	43.36 ± 0.81
8	9	1	0	1	44.86 ± 0.75
13	10	0	0	0	45.75 ± 0.97
2	11	1	−1	0	43.67 ± 0.88
15	12	0	0	0	46.26 ± 0.97
9	13	0	−1	−1	43.01 ± 1.33
7	14	−1	0	1	43.68 ± 0.80
10	15	0	1	−1	43.46 ± 1.16

* Each value represents mean ± SD (n = 3).

The statistical significance of the model equation was evaluated by an F-test ANOVA, as presented in Table 3. The high F-value (25.78) and a low p-value (0.0011) indicate that the model was highly significant and suitable for this experiment. The high coefficient of determination (R^2 = 97.89%) further demonstrates a strong correlation between the predicted and experimental values [21]. In the regression model, the secondary terms BC, A^2, B^2, and C^2 were highly significant ($p < 0.01$). Based on the comparison of F-values, the factors were ranked in order of importance as follows: C > A > B. Additionally, the lack of fit was non-significant ($p = 0.6674$), suggesting the model is adequate. This is consistent with the findings of Tran et al. (2021) [25], who also observed a non-significant lack-of-fit ($p > 0.05$) in their RSM-based optimization of ultrasonic-assisted extraction (UAE) of triterpenoids from Vietnamese red *Ganoderma lucidum*.

Table 3. ANOVA table of the response surface quadratic model analysis of variance table.

Source	Sum of Squares	Df	Mean Square	F-Value	p-Value
Model	24.47	9	2.72	25.78	0.0011 *
A-A	1.30	1	1.30	12.36	0.0170 *
B-B	0.9870	1	0.9870	9.36	0.0281 *
C-C	2.69	1	2.69	25.52	0.0039 *
AB	0.0529	1	0.0529	0.5015	0.5105 ^{ns}
AC	0.0342	1	0.0342	0.3245	0.5936 ^{ns}
BC	1.73	1	1.73	16.39	0.0098 *
A^2	8.76	1	8.76	83.02	0.0003 *
B^2	7.39	1	7.39	70.09	0.0004 *
C^2	4.13	1	4.13	39.15	0.0015 *
Residual	0.5274	5	0.1055		
Lack of Fit	0.2532	3	0.0844	0.6155	0.6674 ^{ns}
Pure Error	0.2742	2	0.1371		
Cor Total	25.00	14			
R^2	0.9789				
Adj-R^2	0.9409				

* $p < 0.05$ = significant; ns = non-significant.

These statistical parameters are in line with previous studies applying Response Surface Methodology for saponin extraction. For instance, Zhang (2025) [26] reported an R^2 of 92.48% when optimizing saponin yield from *Camelia oleifera*, while Liang (2024) Liang, Guan, Lv, Yang, Zhang, Zhao, Zhao and Chen [17] observed an R^2 of 92.48% for *Panax notoginseng* and *Panax quinquefolium* under similar experimental designs. The higher R^2 value (97.89%) obtained in the present study suggests a superior model fit, indicating high predictive accuracy and minimal unexplained variation.

Model adequacy checking was performed by comparing predicted and actual values, as illustrated in Figure 3. The close alignment of the data points to a straight line indicates a satisfactory agreement between the real and predicted results, confirming the suitability of the model.

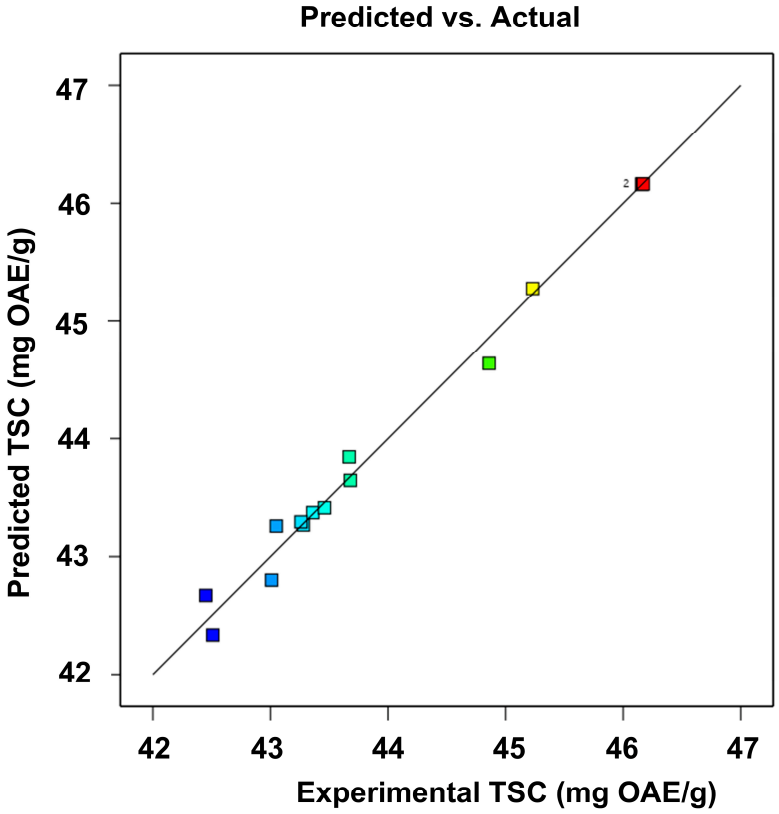


Figure 3. Correlation between predicted and experimental for TSC.

3.2.6. Combined Influence of Extraction Factors on the Extraction of Saponins

The influence of key factors on the saponin red sage root is shown in Figure 4. As seen in Figure 4A,B, the saponin extraction rapidly increased with an increase in ethanol volume in the 1 g sample, reaching a maximum at a ratio of around 40:1 mL/g. However, as the volume of ethanol continued to increase, the saponin content began to decrease. This could be attributed to an initial increase in the concentration gradient between the solvent and the raw material at higher liquid-to-solid ratios, which enhances the dissolution rate of saponins. Conversely, an excessively high liquid-to-solid ratio might negatively impact the cavitation effect, consequently reducing the overall saponin extraction yield [27].

As illustrated in Figure 4A,C, the saponin extraction yield rose with increasing temperature within a certain range. Beyond approximately 60 °C, however, the extraction yield began to decline as the temperature continued to rise. An increase in temperature generally enhances the diffusion coefficient of saponins in the solvent, thereby facilitating its extraction from sage root. Nevertheless, when the temperature exceeds a certain threshold, the structure of saponins may be compromised, leading to a reduction in extraction efficiency. A similar behavior was found for the recovery of saponins from *Polyscias fruticosa* roots, with an optimal extraction temperature of 60 °C [20,21], suggesting the need for careful control of the extraction temperature to preserve saponin integrity.

As regards the effect of time, Figure 4A,C show that an optimal extraction time of about 3 h maximizes the extraction efficiency. This result agrees with that of another study [21] and can be explained by 3h being a sufficient time for saponins' release from the

materials. As the extraction time increased above 3 h, the yield of total saponins reduced, which implies negative reactions causing the yield decline [21].

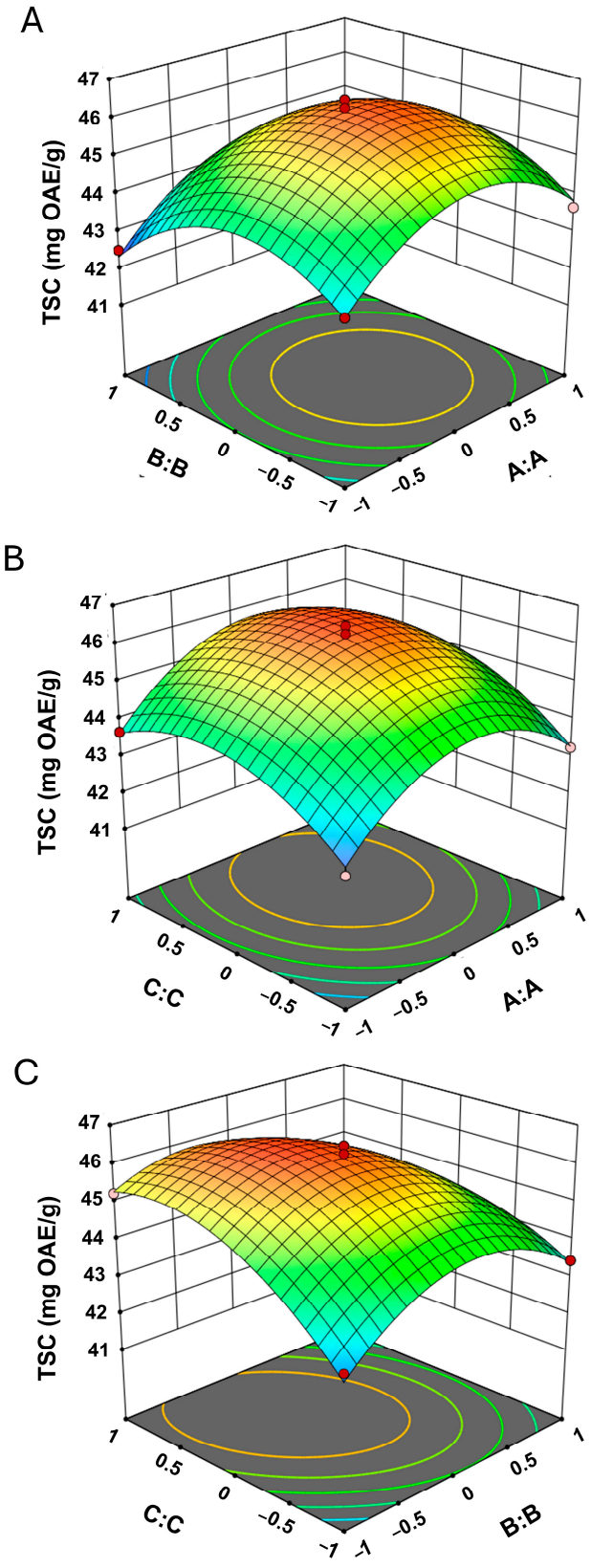


Figure 4. Response surface plots showing the interaction effects of (**A**) ethanol-to-material ratio and temperature, (**B**) ethanol-to-material ratio and time, and (**C**) temperature and time on TSC from *Salvia miltiorrhiza* roots.

Collectively, these results highlight that optimal extraction conditions are crucial to balance efficient solute mass transfer and prevent degradation reactions. These findings are in line with fundamental mass transfer theories in solid–liquid extraction, where extraction efficiency is controlled by the interplay between solute diffusion, solvent saturation, and the thermal stability of target compounds [28,29].

3.2.7. Model Prediction and Validation

The characteristics of the response surfaces and contour plots, as presented in Figure 5, indicate that the recovery of saponins from *red sage* root can be optimized through the judicious selection of extraction conditions. Numerical maximization of the response variable was performed using the gradient descent method, yielding the following results: A = 0.1313, B = −0.1919, C = 0.3333, in terms of natural variables, 41.31:1 (mL/g), extraction temperature = 58.08 °C, and time = 3.17 h. The corresponding maximum extraction yield was 47.71 ± 0.15 mg/g, which is consistent with the predicted yield of 46.32 mg/g. As a result, the model was considered to be accurate and reliable for predicting saponin yield.

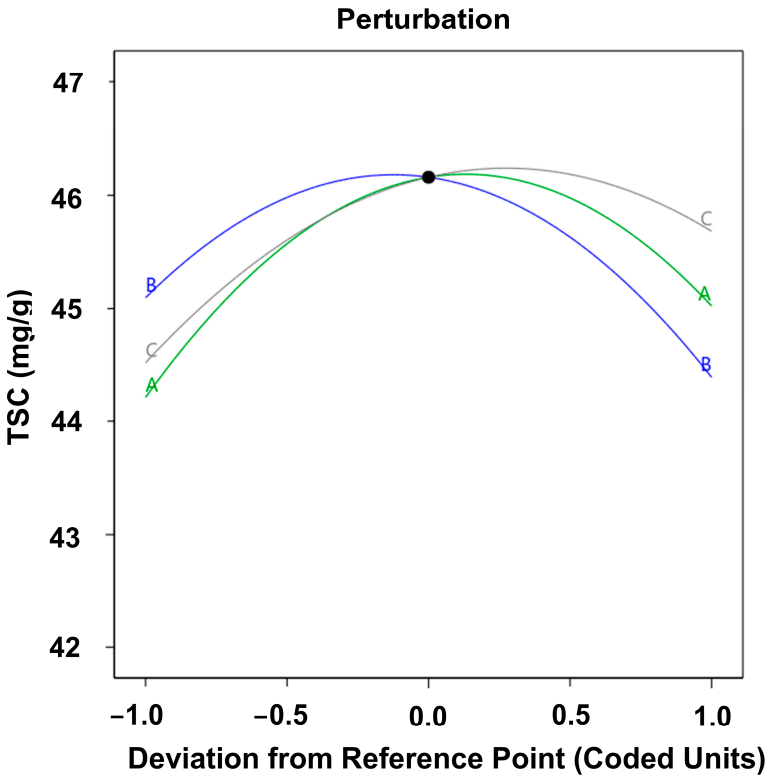


Figure 5. Perturbation plot showing the influence of extraction parameters on TSC.

The relatively low deviation between the experimental and predicted yields reflects the robustness of the Box–Behnken design and the suitability of Response Surface Methodology (RSM) for modeling complex multivariable extraction processes. The narrow standard deviation also suggests good reproducibility under optimized conditions.

Notably, the TSC yield of red sage was found to be considerably higher than that of *Camellia fascicularis* leaves (36.8 mg/g) [21] and *Polyscias fruticosa* Roots (41.24 mg/g) [20]. This indicates that red sage roots are a potential source of saponin compounds. This comparison highlights the superior saponin content of *S. miltiorrhiza* roots, suggesting a greater source for pharmaceutical or nutraceutical development.

3.2.8. DPPH Scavenging Activity

The DPPH radical scavenging activity of the reducing power was measured to evaluate the antioxidant activities of red sage root extracts and ascorbic acid (Figure 6). The sample rapidly inhibited DPPH radicals by up to 61.14% at a concentration of 20 μg/mL. The scavenging activity of red sage extract was approximately 3.38 times lower than that of ascorbic acid, with IC_{50} values of 16.28 and 4.81 μg/mL, respectively. Our study demonstrates that *Salvia miltiorrhiza* root extract, obtained under optimized extraction conditions, exhibits significantly enhanced DPPH radical scavenging activity compared to extracts derived from 70% ethanol extraction (IC_{50} of 23.45 μg/mL) [30]. These findings indicate that employing RSM for optimization is highly effective in maximizing the extraction of bioactive compounds with antioxidant properties from *Salvia miltiorrhiza*. The antioxidant activity of the *S. miltiorrhiza* extract was superior to that of several other plant extracts reported in previous studies. Specifically, the DPPH radical scavenging activity of red sage extract was 12.82 times higher than that of *Sida rhombifolia* leaf extract (IC_{50} of 208.63 μg/mL) [31]. The IC_{50} of the *S. tomentosa* aqueous methanol extract was 18.7 μg/mL, which was slightly higher than *S. miltiorrhiza* extract [32]. Moreover, the IC_{50} red sage extract was significantly lower than that of both the total saponin fraction (440 ± 49 μg/mL) and the crude extract (181 ± 34 μg/mL) derived from *Chlorophytum borivilianum* tubers, which exhibited a higher total saponin content (196.28 mg DE/g) [33]. These findings reinforce the significance of red sage root extract as a valuable natural antioxidant for future applications in the functional food, cosmetic, and pharmaceutical industries.

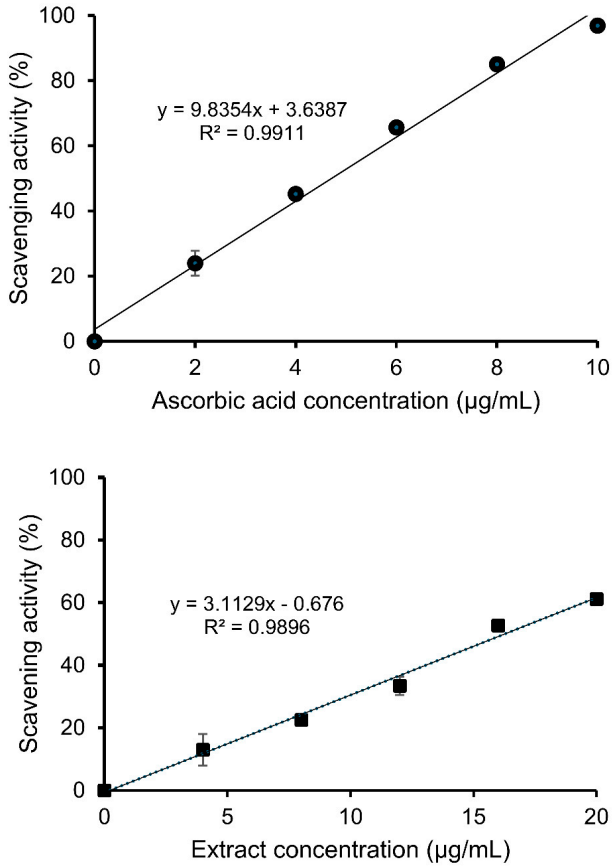


Figure 6. DPPH radical scavenging activity of *Salvia miltiorrhiza* extract and ascorbic acid. The experiment was conducted in triplicate (n = 3).

3.2.9. Cytotoxicity of Red Sage Extract on HepG2

The cytotoxicity of the crude extract of red sage root on HepG2 cell lines was determined through SRB assay. As shown in Table 4, the crude extract of red sage (100 μg/mL) notably inhibited 61.79% of HepG2 cells. Ly et al. [34] also mentioned that plant extract containing saponins exhibited antitumor activity against HepG2. Ethanol extract of *Xanthium strumarium* L. (100 μg/mL) inhibited 73.27% of HepG2 cell proliferation. Furthermore, several studies have also proved that *S. miltiorrhiza* extract presents a potential source for inhibiting HepG2 [35–37]. Thus, red sage extract and its total saponin content exhibited moderate cytotoxicity.

Table 4. Anticancer activity of *Salvia miltiorrhiza* extract against HepG2.

Samples	Concentration (μg/mL)	Inhibition Rate
Salvia miltiorrhiza extract	100	61.79 ± 3.57
Camptothecin	0.01	56.82 ± 2.13

Each value represents mean ± SD (n = 6).

4. Conclusions

This study employed Response Surface Methodology to optimize saponin extraction from *Salvia miltiorrhiza* roots, improving both yield and biological activity. Through the systematic investigation of extraction temperature, time, solvent concentration, and solid-to-liquid ratio, significant enhancements in saponin recovery were achieved. In vitro assessments confirmed the potent antioxidant and anticancer activities of the extracted saponins. These findings demonstrate not only the effectiveness of RSM in optimizing natural product extraction but also the significance of *S. miltiorrhiza* as a sustainable source of multifunctional bioactive compounds that have the potential to improve health. The methodological approach presented here offers a reproducible and scalable strategy suitable for use in the pharmaceutical and nutraceutical industries. Future studies should explore the in vivo efficacy, safety, and bioavailability of the optimized extract. These results affirm the efficacy of RSM in optimizing natural product extraction and underscore the pharmacological value of *S. miltiorrhiza* for potential therapeutic and nutraceutical development.

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